

Excretion of Organic Matter during an Experimental Phytoplankton Bloom Followed Using *o*-Nitrophenol as an Electrochemical Probe*

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Extracellular excretion of the surface active organic matter during a nutrient enriched phytoplankton culture growth was studied using *o*-nitrophenol (ONP) as an electrochemical probe. Changes in the hydrophilic and hydrophobic acid fractions, as compared to the unfractionated seawater sample, were followed during the twelve days of the experiment. Diatoms were the dominant phytoplankton species growing during the experiment. It was observed that the enrichment of natural seawater by nutrients caused increased concentrations of both polysaccharides and proteins, in dissolved and particulate phases. At the end of the experiment, polysaccharides were the dominant adsorbable organic matter on the mercury electrode according to the changes of the electrochemical characteristics of the ONP probe. The excreted polysaccharides were weakly acidic. Their estimated concentration was equivalent to 1.2 mg dm^{-3} as neutral dextran T-500 or 3 mg dm^{-3} as weakly acidic hyaluronic acid.

INTRODUCTION

The biological activity of an aquatic ecosystem depends on the rate of the primary production by photosynthetic organisms. Phytoplankton activi-

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ties are, in turn, affected by the physical environment (temperature, pH, light) and by the kinds and concentrations of nutrients available.^{1,2}

The driving force of biogeochemical cycles in the seas is the organic matter derived from photosynthetic activities. A knowledge of the factors influencing algal productivity is thus of importance for understanding and predicting their influence on the biogeochemical cycles. From this aspect, a focus of interest of many marine scientists is to find the key factor for the appearance of mucilages^{3,4} after extensive phytoplankton blooms, which are composed mostly of polysaccharides,⁵ and can represent an ecological as well as an economic problem. Polysaccharides are the dominant component of reactive material in the marine environment, supporting perhaps most of the heterotrophic activity in surface waters.⁶

Phytoplankton exudates constitute the main source of naturally occurring organic matter in seawater. A large fraction of this organic matter is surface active, and represents the major part of surfactant activity in the sea.^{7,8}

Dissolved organic substances and colloidal macromolecules may have surface active properties which enable them to adsorb at natural phase boundaries.^{9,10} On the other side, adsorption of organic substances on the mercury electrode can be used for their determination and characterization by different electrochemical methods.¹¹

The aim of this work was to study, using *o*-nitrophenol (ONP) as an electrochemical probe, the production of surface active organic material during the evolution of a phytoplankton bloom.

Electrochemical study of organic matter in the XAD-8 fractions of different hydrophobicity during a semi-field experiment of phytoplankton growth was performed as part of the project Production and Accumulation of Labile Organic Matter in the Adriatic (PALOMA), called the Palex experiment. Here, we will present the results of the Palex III experiment which lasted from March 27 to April 3, 1996. In this experiment, the chlorophyll *a* standing crop of phytoplankton was in the range of ≈ 1 to $12 \mu\text{g dm}^{-3}$, with the maximum occurring on the fifth day of the experiment, after which its concentration was slowly decreasing. Pigment fucoxanthin, which is a useful biomarker for the presence of diatoms, reached a maximum value ($\approx 14 \mu\text{g dm}^{-3}$) on the seventh day of the experiment, which was just slightly higher than that on the fifth day. Chlorophyll *a* concentration reached the highest concentration on the last day of the experiment, indicating a pronounced senescence of the present phytoplankton.¹²

Characterization of Surface Active Substances Using o-Nitrophenol as an Electrochemical Probe

In the presence of adsorbed layers of organic matter on the mercury electrode, electrochemical characteristics of *o*-nitrophenol (ONP) change in

significantly different ways, depending on the different properties of organic substances, such as hydrophobicity, acidity, molecular weight, *etc.* Based on this phenomenon, a method was developed for rough characterization of organic matter in seawater samples by measuring the changes in the ONP peak¹³ and prepeak¹⁴ heights, and shifts of the peak potential.¹³

Generally, by increasing the concentration of surface active substances, the ONP height continuously decreases, with the peak being shifted towards more negative potentials, until the maximum adsorption of the present surface active substances is achieved.

The height of the prepeak is extremely sensitive to the type of organic matter adsorbed on the mercury electrode at the pH of natural seawater.¹⁴ The ONP prepeak increases in the presence of adsorbed layers of negatively charged organic substances, such as humic and fulvic acids and proteins, while in the presence of adsorbed uncharged polysaccharides, represented by model substances of dextran type, there is no increase in the ONP prepeak height. This is a good basis for differentiation of these substances in natural seawater samples. However, this differentiation is not satisfactory in the case of acidic polysaccharides since in their presence the ONP prepeak height increases in the same way as for other negatively charged polyelectrolytes.

Substantial improvement in the electrochemical characterization of organic matter in seawater samples using the ONP probe was achieved by fractionation of organic substances on XAD-8 resin.¹⁵ Advantages of fractionation are the separation of hydrophobic acid from hydrophilic organic materials, which makes it possible to distinguish between the adsorption effects of acidic polysaccharides and those of fulvic and humic acids, as well as to obtain a better distinction of proteins from other organics.

EXPERIMENTAL

Methods

Electrochemical Measurements

Surface active substances in seawater samples and fractions were determined by the phase sensitive alternating current (a.c.) voltammetry using ONP as an electrochemical probe, as described in previous papers.^{13,14} Oxidation-reduction processes of ONP were measured by the in-phase mode, at a hanging mercury drop electrode. All measurements were performed using a EDT-ECP-100 Modular Research Polarograph, connected to an ECP-110 unit (EDT) and to a Hewlett-Packard 7045 A recorder. The frequency of the a.c. voltage was 170 Hz, and the p-p amplitude 10 mV. The scan rate was 20 mV s⁻¹. Surface active organic material was adsorbed on the working electrode at a potential of -0.35 V *vs.* Ag/AgCl reference electrode, prior to the potential scan. Accumulation of organic substances was performed by stirring the sample for 1, 3 and 10 min. All voltammetric measurements of ONP include two

scans: the first scan, recorded from the accumulation potential (-0.35 V) up to -0.89 V, represents the main ONP peak, and the second scan, recorded immediately after the potential was returned to the initial potential at the same mercury drop electrode, represents the ONP prepeak.

Fractionation of Seawater Samples

Fractionation of organic matter into hydrophobic and hydrophilic fractions was performed using XAD-8 resin based on the method described by Vojvodić *et al.*¹⁶ and references therein. Hydrophobic and hydrophilic fractions obtained on the XAD-8 resin are operationally defined as follows. The hydrophobic basic and neutral organic substances are sorbed on the resin at the pH of seawater and hydrophobic acids are sorbed at pH = 2, while hydrophilic organic compounds are non-sorbable, and remain in the final effluent.

In the first step, hydrophobic neutral and basic compounds were separated from the seawater by sorption on the XAD-8 resin at the pH of seawater. The solution remaining after sorption (Fraction I), composed of hydrophobic acid and hydrophilic organic materials, was acidified to pH = 2, and passed through the second XAD-8 resin in order to remove the hydrophobic acid compounds. Hydrophilic compounds (Fraction II), which are not sorbed, remained in the final effluent. The pH of the hydrophilic fraction was adjusted to pH = 8.4 before measurement with the ONP probe. Hydrophobic acid compounds were eluted with 0.1 M NaOH. In this fraction (Fraction III), the pH and ionic strength were adjusted to 0.55 M NaCl and pH = 8.4, and measured with the ONP probe. Elution of the hydrophobic acid fraction was not quantitative. All samples were treated in the same way, which enabled comparison of the qualitative composition of different samples. Seawater samples were filtered through a 0.7 μm pore size Whatman filter before fractionation.

ONP and hyaluronic acid (Sigma), dextran T-500, $M_r \approx 500\,000$ (Serva), HCl and NaOH (Kemika, Croatia) were used without further purification.

DOC Measurements

Determination of DOC was carried out by a Shimadzu DOC analyzer provided with the Shimadzu High Sensitive TOC Catalyst (Japan).

Palex III Experiment

The Palex III experiment was performed in such a way that the natural plankton assemblage from the original seawater sample, collected at an offshore station in the north Adriatic at a 1 m depth (initial seawater sample 82-C-1), was pre-screened through 200 μm mesh to remove zooplankton grazers and thereafter enriched by addition of nutrients (G-1 samples), whose final concentrations were 0.6 $\mu\text{mol dm}^{-3}$ phosphate, 5.1 $\mu\text{mol dm}^{-3}$ nitrate and 1.8 $\mu\text{mol dm}^{-3}$ ammonium as nitrogen, and 10.6 $\mu\text{mol dm}^{-3}$ silicate, after which the experiments were carried out in large transparent nalgene bottles, incubated in the sea at a 2 m depth.

In the framework of this experiment, investigation of the chemical nature of dissolved organic matter was performed by various analytical methods. Nutrients and carbon changes, evolution of the primary production, as well as microbiological activity were also analyzed. Samples were analyzed every few days during the twelve days of the Palex III experiment.

RESULTS AND DISCUSSION

Electrochemical characteristics of the ONP probe, for different accumulation times, obtained in seawater samples taken on different days during the enrichment experiment are presented in Table I. Data are given for non-

TABLE I

Electrochemical characteristics of the ONP voltammetric peak for the nonfiltered and filtered seawater samples and fractions obtained for different accumulation times (1, 3 and 10 minutes)

Sample	Date	$\Delta E/\text{mV}^a$			i_p/i_{p0}^b			i_A/i_{A0}^c		
		1	3	10	1	3	10	1	3	10
82-C-1 NF ^e	March 22, 1996	15	42	103	0.82	0.67	0.40	1.8	2.8	2.2
82-C-1 F ^f		11	35	90	0.88	0.69	0.51	1.6	2.6	3.2
Fraction II		2	14	53	0.90	0.73	0.56	1.3	1.8	1.8
Fraction III		18	74	131	0.70	0.58	0.59	1.7	1.7	1.3
86-G-1 NF	March 26, 1996	12	39	82	0.87	0.72	0.59	1.5	2.5	3.6
86-G-1 F		5	24	55	0.86	0.76	0.71	1.5	2.1	3.9
Fraction II		9	16	56	0.95	0.72	0.53	1.5	2.4	3.3
Fraction III		7	35	91	0.82	0.59	0.49	1.3	1.9	2.0
87-G-1 NF	March 27, 1996	13	46	85	0.81	0.70	0.55	1.7	3.2	4.1
87-G-1 F		8	21	52	0.90	0.77	0.74	1.5	2.4	3.7
Fraction II		7	28	79	0.85	0.69	0.60	1.6	2.1	1.8
Fraction III		15	104	130	0.73	0.54	0.46	1.6	1.4	1.3
89-G-1 NF	March 29, 1996	48	56	88	0.54	0.50	0.35	1.6	1.5	1.1
89-G-1 F		20	34	61	0.80	0.63	0.39	1.6	1.5	1.0
Fraction II		9	25	59	0.91	0.68	0.38	1.5	2.0	1.1
Fraction III		27	80	127	0.59	0.50	0.48	2.3	2.5	1.7
92-G-1 NF	April 01, 1996	32	55	55	0.58	0.39	0.41	1.5	1.6	1.4
92-G-1 F		16	35	44	0.86	0.69	0.50	1.5	2.4	1.3
Fraction II		19	38	56	0.74	0.47	0.36	1.4	1.3	1.6
Fraction III		20	68	70	0.72	0.52	0.54	1.4	1.6	1.5
94-G-1 NF	April 03, 1996	50	53	62	0.52	0.51	0.46	1.2	1.1	1.0
94-G-1 F		25	41	55	0.78	0.57	0.47	1.8	1.1	1.2
Fraction II		22	41	42	0.75	0.50	0.44	1.7	1.4	2.9
Fraction III		50	116	145	0.50	0.50	0.60	1.1	1.1	D ^d

^aShift of the peak potential of the ONP voltammetric peak; ^bnormalized peak current of ONP, i_{p0} is the peak current without accumulation and i_p is the peak current after certain accumulation time; ^cnormalized prepeak current of ONP (second scan), i_{A0} is A prepeak current without accumulation and i_A is the prepeak current after certain accumulation time; ^dprepeak disappears at completely covered electrode; ^enonfiltered sample; ^ffiltered sample.

filtered and filtered seawater samples, as well as for the hydrophilic fraction (Fraction II), and the hydrophobic acid fraction (Fraction III) obtained by using XAD-8 resin. Typical voltamograms of ONP in the seawater samples, filtered and nonfiltered, and the hydrophilic fraction, are presented in Figure 1 for the initial seawater sample, and in Figure 2 for the sample taken on the twelfth day of the experiment.

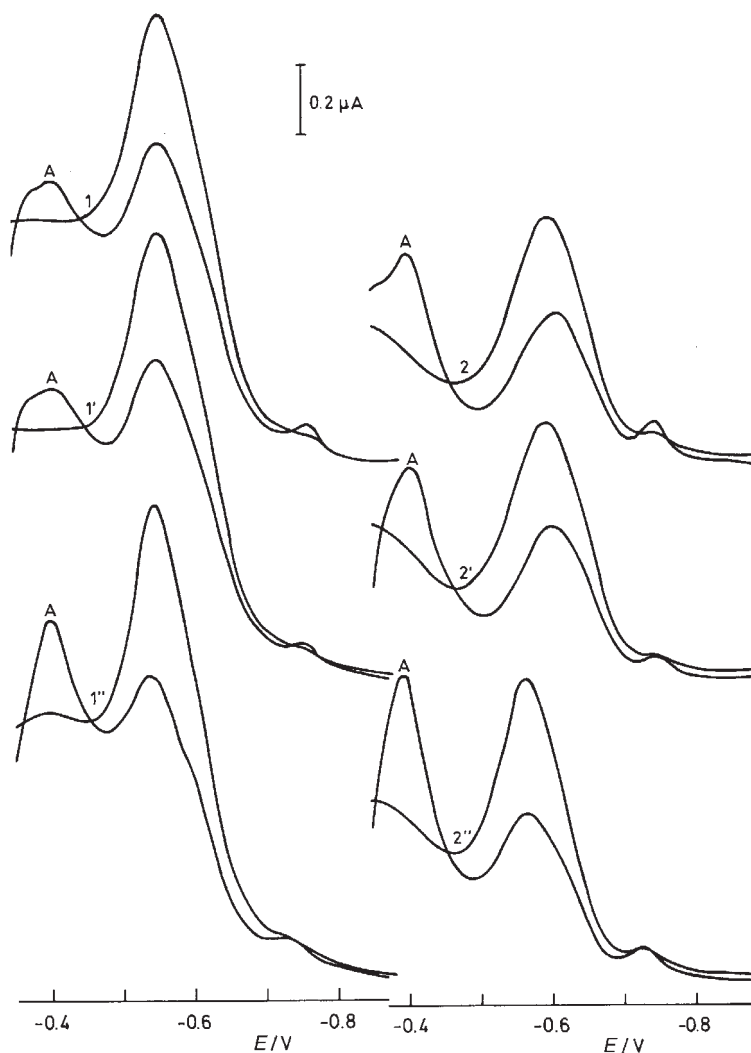


Figure 1. A.c. voltamograms of 10^{-4} M ONP in the initial seawater sample, 82-C-1, March 22, 1996 (1, 2), filtered sample (1', 2'), and hydrophilic fraction (1'', 2''). Accumulation time: 0 (1, 1', 1'') and 3 min (2, 2', 2''). Curve (A) second scan at the same mercury drop, recorded immediately from potential -0.35 V.

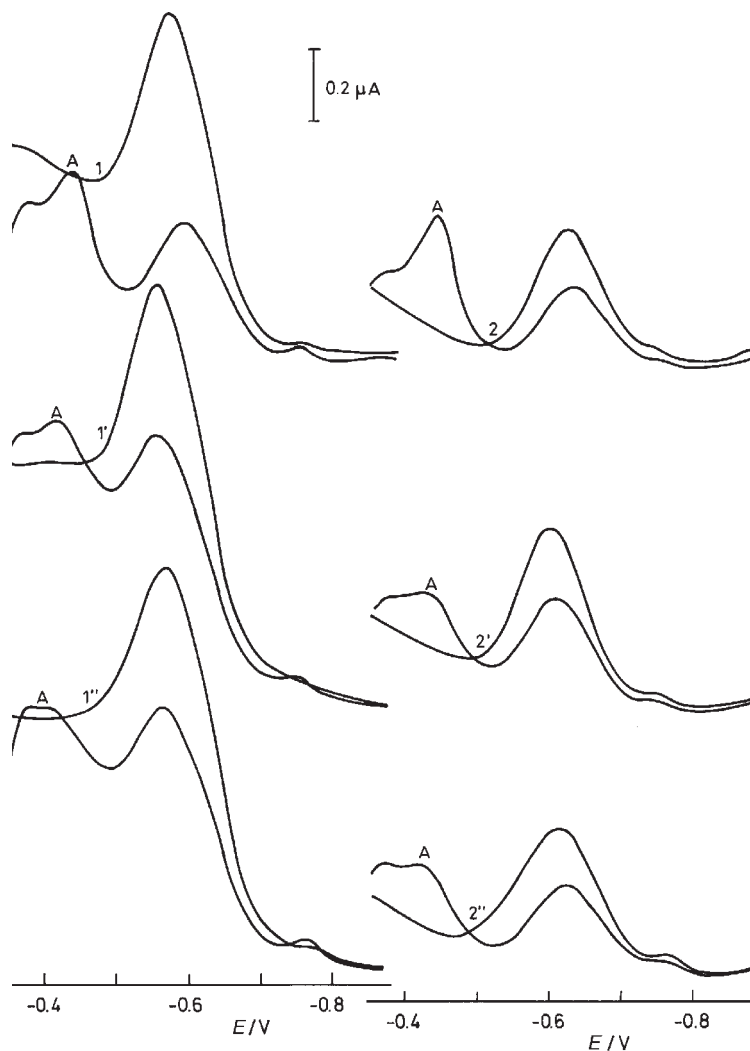


Figure 2. A.C. voltamograms of 10^{-4} M ONP in the enriched seawater sample after twelve days, 94-G-1, April 03, 1996 (1, 2), filtered sample (1', 2'), and hydrophilic fraction (1'', 2''). Accumulation time 0 (1, 1', 1'') and 3 min (2, 2', 2''). Curve (A) second scan at the same mercury drop, recorded immediately from potential -0.35 V.

As regards the initial seawater sample (82-C-1) (Figure 1), from the change in electrochemical characteristics of the ONP probe, a significant shift of peak potential, 90 mV for 10 min of accumulation, from a big increase in prepeak height, at the most 3.2 times, and from the specific shape of peaks specially visible for a longer accumulation time, it can be concluded that the dominant organic material in this sample are hydrophobic acidic substances,

very probably a mixture of proteins and refractory fulvic acid material. From the calibration curves for the protein albumin and naturally isolated fulvic acid reported in our previous papers,^{13,14,15} we estimated the effects for this filtered sample as corresponding approximately to 0.5 mg dm⁻³ albumin and 0.4 mg dm⁻³ fulvic acid.

Comparing the electrochemical characteristics of the ONP probe added to nonfiltered and filtered sample of the initial seawater, it can be concluded that the same type of material was present in both samples, with somewhat higher concentrations of organic matter in the nonfiltered sample.

As regards the hydrophilic fraction, from the small change of electrochemical characteristics of the ONP probe, we could conclude the presence of a relatively low concentration of surface active substances in this fraction, most likely corresponding to several types of polysaccharides. The increase in the prepeak height of the ONP probe with increasing adsorption time points to the presence of acidic polysaccharides. In order to estimate the approximate concentration level of polysaccharides in the hydrophilic fraction, we have used two model substances, dextran T-500 and hyaluronic acid. The voltammetric characteristics of ONP (peak height and the shift of peak potential) in the presence of increasing concentrations of these model substances are presented in Figure 3. Using these curves for calibration, we could estimate an equivalent effect of about 0.65 mg dm⁻³ dextran or 1.0 mg dm⁻³ hyaluronic acid.

On the fourth day of experiment, after addition of nutrients, to sample 86-G-1, the electrochemical characteristics of the ONP probe were found to respond to a mixture of hydrophobic acid and hydrophilic organic material. The concentration of polysaccharides was not changed significantly in the hydrophilic fraction, as compared to sample 82-C-1. Estimated concentrations of polysaccharides in this sample could be 0.6 mg dm⁻³ dextran or 1.4 mg dm⁻³ hyaluronic acid.

Nonfiltered sample 86-G-1 is composed of hydrophobic acid and hydrophilic organic material, in concentrations which are very slightly higher in comparison with those of the filtered sample.

In sample 87-G-1, the ONP probe showed almost the same electrochemical characteristics as in sample 86-G-1, when the samples were compared before fractionation. In the hydrophilic fraction, an increase in organic material was visible considering the bigger shift of potential and the decrease in ONP main peak height. According to the changes in the electrochemical characteristics of ONP in the hydrophobic acid fraction, an increase in the concentration of very probably dissolved proteins was noticed.

From the bigger effects of SAS on the electrochemical characteristics of the ONP probe obtained for the nonfiltered than for the filtered 87-G-1 sample, we can conclude that surface active substances in the particulate or-

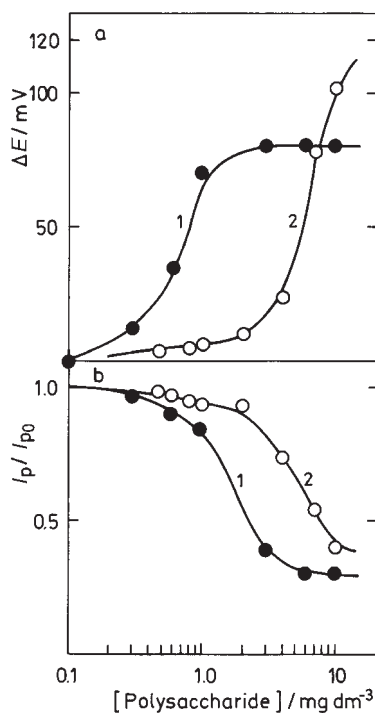


Figure 3. (a) Dependence of the shift of the ONP peak potential on the concentration of dextran T-500 (1) and hyaluronic acid (2). (b) Dependence of the normalized ONP peak current on the concentration of dextran T-500 (1) and hyaluronic acid (2). Accumulation time 1 min.

ganic matter give a relatively big contribution to the surfactant activity in the nonfiltered sample.

The same was observed, to a larger extent, in the nonfiltered sample taken on the seventh day of the experiment (89-G-1).

In a similar experiment of the production and utilization of DOC during the experimental diatom bloom,¹⁷ it was shown that chlorophyll *a* reached the maximum value on the eighth day, while inorganic nutrients, phosphate and nitrate concentrations decreased to near-zero levels on days 6 and 7. At the same time, accumulation of DOC was low during the bloom formation until the seventh day, but increased considerably between days 7–10. It is also known that extracellular carbohydrates are liberated by phytoplankton during all stages of their growth, but the major portion is released into the surrounding environment during the stationary growth stage.¹⁸ As the population of diatoms, in our experiment, reached the stationary phase on the fifth day, the dominant adsorption behaviour of surface active substances from the hydrophilic fraction, which is mainly composed of carbohydrates,

observed after the seventh day (samples 92-G-1 and 94-G-1), seems to be very reasonable, and is in agreement with both previous references.^{17,18}

The ONP probe investigation of seawater sample 94-G-1 from the Palex III experiment is illustrated in Figure 2. The electrochemical characteristics of the ONP in the filtered seawater sample, a significant decrease in the main peak, and the small shift of the peak potential even in 10 min of accumulation, which are almost the same as for the hydrophilic fraction itself, point to the conclusion that polysaccharides prevailed in the adsorption layer at the mercury electrode.

Based on the smaller increase of the prepeak height of ONP in samples 92-G-1 and 94-G-1, as compared to the samples from the earlier phase of the experiment, we could conclude on the increasing contribution of neutral polysaccharides in the later phase of the experiment.

Evaluating from the calibration curves for hyaluronic acid, we estimated the concentration of that type of polysaccharide at approximately 3 mg dm^{-3} and about 1.2 mg dm^{-3} for dextran.

In the nonfiltered sample 94-G-1, the presence of a very high concentration of the same type of very hydrophilic organic material was detected as well. For this sample, a complete coverage of the electrode surface was reached in 30 s of accumulation, while in the filtered sample it occurred after ≈ 3 min of accumulation. For the nonfiltered sample, the concentration of the dominant hydrophilic organic matter was estimated at $\approx 7.0 \text{ mg dm}^{-3}$ expressed as polysaccharide hyaluronic acid.

The increased concentration of hydrophobic acid organic substances was suggested based on the rather big shift of peak potential, and the characteristic shape of the ONP peak in Fraction III of sample 94-G-1. We suppose that the increased concentration of proteins which constitute this fraction,¹⁵ was the main reason for the increased changes of the ONP electrochemical characteristics in the last sample(s) of the experiment, as compared to the initial seawater sample, 82-C-1. Namely, it is not reasonable to expect that refractory material like fulvic or humic acids, which could constitute this fraction, could increase in concentration during the twelve days of this experiment. Investigations of the formation of humic and fulvic acids in degrading diatom debris showed that these acids are formed in four steps, which occurred in time periods from weeks to over many hundred years.¹⁹

The presence of proteineous organic matter was not visible in the measured nonfractionated seawater sample because of the masking effects due to the presence of polysaccharides. Despite the fact that alkaline elution was not quantitative, which constitutes a problem in estimating the total concentration of SAS in these fractions, a comparison of hydrophobic acid fractions of different seawater samples is possible since all hydrophobic acid fractions were treated in the same way.

Polysaccharides are hydrophilic by nature, and using the XAD-8 resins they transform into the hydrophilic fraction, 100% for neutral and more than 80% for acidic ones.¹⁶ They may possess surface active properties, and thus adsorb at the mercury electrode as well. Surface active properties of polysaccharides produced by diatoms were confirmed by Wilson and Collier.⁷

Benner *et al.*⁶ found a relatively high abundance of polysaccharides ($\approx 50\%$ from all dissolved organic matter) in surface waters of the open North Pacific Ocean, with a decreased level of $\approx 25\%$ in deeper waters. It was found, that in surface waters, high molecular weight polysaccharides (>1000 daltons) made up an important part of the labile DOM. Thus, polysaccharides appeared to be the most abundant and reactive components of the dissolved organic matter in seawater.

Concentrations of polysaccharides, as the most likely surface active substances present in the hydrophilic fraction during the experiment of phytoplankton development, were evaluated from the calibration curves for the acidic polysaccharide hyaluronic acid and neutral polysaccharide dextran, as presented in Table II. The content of dissolved organic carbon in the same fraction is also presented. Neither of the two polysaccharides used for the calibration is an ideal model substance, mostly due to the fact that the type of natural carbohydrates was changing during the experiment, especially in acidity. Nevertheless, it is evident that the effect of surface active substances upon the ONP probe was increasing from the first to the twelfth day of the experiment. If the estimated concentrations of polysaccharides are compared with the DOC values, taking into account that polysaccharides have an average content of 40–50% organic carbon, surface active polysaccharides seem to represent the dominant component of organic car-

TABLE II

DOC values and estimated concentrations of surface active substances (SAS) for the hydrophilic fraction (Fraction II) in the enrichment experiment*

Sample	DOC	SAS/mg dm ⁻³	
	mg dm ⁻³	dextran	hyaluronic acid
82-C-1 Fraction II	0.86	0.65	1.0
86-G-1 Fraction II	0.68	0.60	1.4
87-G-1 Fraction II	0.66	0.70	1.6
89-G-1 Fraction II	0.84	0.65	1.7
92-G-1 Fraction II	0.80	1.20	2.7
94-G-1 Fraction II	1.24	1.20	3.0

* For surface active substances, calibration curves for dextran T-500 and hyaluronic acid were used.

bon at the end of the experiment, while in the initial seawater sample at the start of the experiment they constitute only a smaller part of dissolved organic carbon.

CONCLUSIONS

In the Palex III experiment, performed in early spring, enrichment of seawater with nutrients (phosphate, nitrate, ammonium, and silicate) caused a bloom of diatoms.²⁰ As a consequence, increased concentrations in both carbohydrates and proteins were detected, especially at the end of the experiment.

Namely, by using ONP as an electrochemical probe, in combination with fractionation of seawater samples by sorption on XAD-8 resin,^{13–15} it was possible to identify the increasing effects of polysaccharides in the hydrophilic fraction as well as in the untreated seawater sample, and the increasing effects of proteins in the hydrophobic acid fraction.

Carbohydrates started to be the dominant surface active substance on the mercury electrode surface after the seventh day of the experiment. The concentration of this organic material increased 2–3 times, using dextran T-500 and hyaluronic acid for calibration. Neither dextran nor hyaluronic acid revealed fully the electrochemical behaviour of natural polysaccharide, found in these samples. Namely, the influence of natural polysaccharides on the electrochemical characteristics of ONP was stronger than that of hyaluronic acid and weaker than that of dextran T-500. Despite this, we tried to evaluate very roughly the concentrations of polysaccharides from the start to the end of the experiment.

The increase in the concentrations of polysaccharides observed during the experiment was in agreement with the expected type of organic matter excreted by diatoms. Diatoms are known to produce polysaccharides in higher amounts than other phytoplankton species into the media where they are growing.^{5,21,22} The acidity of carbohydrates was changing throughout the experiment, from more acidic at the beginning to less acidic at the end. This was concluded from the change in the prepeak height of the ONP probe obtained in the hydrophilic fraction.

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SAŽETAK

Praćenje izlučene organske tvari tijekom eksperimentalnog cvata fitoplanktona uporabom *o*-nitrofenola kao elektrokemijske probe

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Uporabom *o*-nitrofenola (engl. kratica ONP) kao elektrokemijske probe praćeno je izlučivanje površinski aktivnih organskih tvari tijekom rasta fitoplanktona u moru obogaćenom hranjivim solima. Tijekom 12-dnevnog eksperimenta također su praćene promjene organske tvari kisele hidrofobne i hidrofilne frakcije u usporedbi s nefrakcioniranim uzorcima morske vode. Tijekom eksperimenta glavna vrsta fitoplanktona koja se razvila bile su diatomeje. Nađeno je da je obogaćenje hranjivim solima dovelo do obogaćenja morske vode polisaharidima kao i proteinima, u otopljenom i partikularnom obliku. Temeljeno na promjeni elektrokemijskih značajki ONP, polisaharidi su bili dominantne površinski aktivne organske tvari. Također je nađeno da su ti polisaharidi bili slabo kiseli. Procijenjene koncentracije polisaharida, na kraju pokusa, iznose $1,2 \text{ mg dm}^{-3}$ tipa neutralnog šećera dekstrana T-500 ili 3 mg dm^{-3} slabo kiselog polisaharida hialuronske kiseline.